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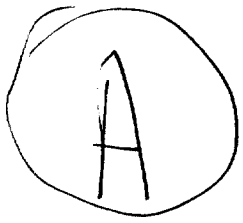

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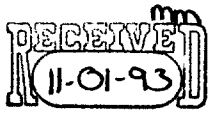

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Document Processing Center (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Attention: 8(e) Notification Coordinator

Subject: Chemical Name: CI Solvent Yellow 14
Test Model: Rat and Mouse Micronucleus Tests

ZENECA Specialties recently received a report from a study to determine the clastogenic potential in rats and mice of one of our chemicals. We believe this information is reportable under Section 8(e) of the Toxic Substances Control Act (TSCA).

CI Solvent Yellow 14 was evaluated for its ability to induce micronucleated polychromatic erythrocytes in the bone marrow of Alp:APfSD male rats and C57BL/6JfBL10/Alpk male mice. A single oral dose was given to groups of five rats and mice at a dose level of 5000 mg/kg, the limit dose. Bone marrow samples were taken 24 and 48 hours after dosing.

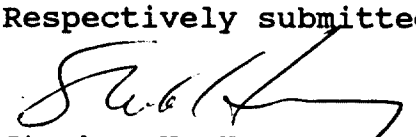
A previous study by Westmoreland and Gatehouse (1991) which is cited in the report concluded that CI Solvent Yellow 14 was positive in the rat bone marrow micronucleus test, but negative in a mouse bone marrow micronucleus test up to dose levels of 2000 mg/kg.

79 pgs.

In the subject study a higher 5000 mg/kg dose was administered and as expected the male rats showed a small but significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control. Likewise the mouse showed a small but significant increase in the incidence of micronucleated polychromatic erythrocytes. We conclude that under the conditions of the tests, CI Solvent Yellow 14 is clastogenic in the rat micronucleus test and weakly positive in the mouse test.

ZENECA Specialties sells this dye to manufacturers of colored plastic materials. The dye is added to the polymer in the extruder at the beginning of the process and exposure is limited to those who charge the reaction vessel. After extrusion the dye is incorporated into the polymeric material. We are aware that this dye in liquid form is used to color gasoline; however, ZENECA Specialties does not sell into this market.

Respectively submitted,



Stephen K. Harvey
Manager, Environment and Product Safety
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CATEGORY B REPORT
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Sponsor: Zeneca Specialties
Sponsor Ref: AD/91/0038
CTL Ref: Y03474/002
CTL Study Nos: SM0613 and SR0614
Copy No: 2

REPORT NO: CTL/T/2835

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

by

K Griffiths
J M Mackay

Approved for Issue: J W Botham
Product Manager

Date of Issue:
02 JUL 1993

I, the undersigned declare that this report constitutes a true record of the actions undertaken and the results obtained in the above study.

J M Mackay (Study Director) ..... 25 June 1993

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT
AND MOUSE MICRONUCLEUS TESTS

The following contributed to this report in the capacities indicated:

K Griffiths - Study Investigator and Cytogenetic Analyst

M D H Ryan - Home Office Licensee

D J Barker - Home Office Licensee

M Greenwood - Statistician

Reviewed by:
B M Elliott 25.6.93
(Head, Regulatory Genetic Toxicology)

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

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CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

SUMMARY

CI Solvent Yellow 14 has been evaluated for its ability to induce micronucleated polychromatic erythrocytes in the bone marrow of Alpk:AP$_f$SD male rats and C57BL/6J$_f$BL10/Alpk male mice. A single oral dose was given to groups of 5 male rats and 5 male mice at a dose level of 5000mg/kg. In each case the dose level used represents the limit dose level of the assay. Bone marrow samples were taken 24 and 48 hours after dosing.

Initial evaluation of the incidence of micronucleated polychromatic erythrocytes observed in 1000 polychromatic erythrocytes per animal in both the rats and mice resulted in some increases over the vehicle control values. Analysis of increased numbers of polychromatic erythrocytes together with a repeat evaluation in both the rat (5000mg/kg) and mouse (2000 and 5000mg/kg) were undertaken in order to clarify these findings. The final interpretation was made from an examination of 6000 polychromatic erythrocytes from all animals studied.

In the rat, considering the data from the combined counts of 6000 polychromatic erythrocytes per animal, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 and 48 hour sampling times in animals dosed with CI Solvent Yellow 14 at 5000mg/kg in both studies.

In the mouse, considering the data from the combined counts of 6000 polychromatic erythrocytes per animal, a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was observed in the first study at the 48 hour sampling time in animals dosed with CI Solvent Yellow 14 at 5000mg/kg. In the second mouse study, small but statistically

SUMMARY - continued

significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 hour sampling time in animals dosed with CI Solvent Yellow 14 at 2000mg/kg, and at the 24 and 48 hour sampling times in animals dosed with CI Solvent Yellow 14 at 5000mg/kg.

Comparison of the percentage of polychromatic erythrocytes in both rat and both mouse studies showed only isolated statistically significant differences between the CI Solvent Yellow 14 and the vehicle control animals. These differences were very small and are considered not to be of any biological significance.

Colouration of the urine from both rats and mice treated with CI Solvent Yellow 14 was observed, indicating that the test material was absorbed and distributed in both species when administered via the oral route.

The test system positive control, cyclophosphamide, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to the vehicle control values, in both mouse and both rat studies, thus demonstrating the sensitivity of the test system to a known clastogen.

Considering all of the above data, it is concluded that CI Solvent Yellow 14, under the conditions of test, is clastogenic in the rat micronucleus test and weakly clastogenic in the mouse micronucleus test.

1. INTRODUCTION

Westmoreland and Gatehouse (1991) have reported CI Solvent Yellow 14 as positive in a rat bone marrow micronucleus test, but negative in a mouse bone marrow micronucleus test up to a dose level of 2000mg/kg. To investigate these published results, a sample of CI Solvent Yellow 14 from Zeneca Specialties was tested for its ability to induce clastogenic effects using mouse and rat bone marrow micronucleus tests up to a limit dose level of 5000mg/kg.

The micronucleus test is capable of detecting the clastogenic effect of a chemical. After chromosomal damage has been induced by a test compound or its metabolites, acentric fragments of chromosomal material lag behind at anaphase. At telophase a large proportion of these fragments is not included in the main daughter nuclei. This can result in the formation of small secondary nuclei or micronuclei.

Micronuclei can be formed in a wide variety of cell types, but in this test system bone marrow erythrocytes are observed because micronuclei can easily be detected in this cell type, since the nucleus proper is extruded during maturation.

A few hours after their last division is completed, erythroblasts expel their nuclei and become polychromatic erythrocytes. The term polychromatic is derived from the reaction of the cell with Romanovsky stains; residues of nucleic acids remain for a short time after the expulsion of the nucleus causing the cell to stain a blue-grey colour, whereas the mature erythrocyte appears pink.

Polychromatic erythrocytes are useful for the detection of clastogenic chemicals because they persist for only 24 hours before maturing into normochromatic erythrocytes. Consequently, any micronuclei in these cells will have been produced at the last mitotic division and their formation will be due to the effects of the chemical in the preceding 48 hours.

The clastogenic potential of CI Solvent Yellow 14 was assessed in the micronucleus assay, following its administration as a single oral dose, as recommended in OECD Guideline 474 (1983). The established clastogen, cyclophosphamide, was used as a positive control in order to demonstrate the sensitivity of the test system. Male animals only were used in these studies as in the published report by Westmoreland and Gatehouse (1991).

All data pertaining to this study are stored in the Archives at Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. A copy of this report is held by the Report Centre at the same address.

The experimental phase of this study was carried out between 3 December 1991 and 23 September 1992. The slides were analysed between 6 January 1992 and 2 March 1993.

2. EXPERIMENTAL PROCEDURES

2.1 Test Sample

The test sample of CI Solvent Yellow 14 (Batch Ref: MIX 68911) was obtained from Zeneca Specialties, St Clair Du Rhone, and was submitted for testing by Zeneca Specialties. It had Sponsor reference AD/91/0038 and was given CTL reference number Y03474/002. The test sample was supplied as a yellow/orange powder with an analysed purity of 86% w/w (Reference: ASG 12268/89).

The test material was stored at ambient temperature in the dark until required and was tested as a suspension in corn oil with no correction for purity.

2.2 Control Chemicals

The positive control, cyclophosphamide, was supplied by Sigma Chemical Company Ltd, Fancy Road, Poole, UK, and was given the CTL reference number Y01259/007. It was dissolved in sterilised physiological saline, CTL reference number Y06538/001 immediately prior to use.

The vehicle control was corn oil, supplied by Central Dispensary, CTL, and was given the CTL reference number Y00790/004.

2.3 Preparation of Dosing Solutions/Suspensions

Dosing suspensions of the test material were prepared in corn oil by homogenisation, the same preparations being used to dose both the mice and rats. Solutions of cyclophosphamide were prepared in physiological saline, CTL reference number Y06538/001. All dosing preparations were administered at a volume of 20ml/kg bodyweight. Fresh preparations were used on each day of dosing.

2.4 Animals and Husbandry

Male C57BL/6J$_f$BL10/Alpk mice in the age range of 10-11 weeks, 7-12 weeks and 6-7 weeks were used for Phases I, II and III of the mouse study respectively.

Male Alpk:AP$_f$SD rats in the age range 5-7 weeks, 7-9 weeks and 5-7 weeks were used for Phases I, II and III of the rat study respectively.

Both the mice and the rats were supplied by the Barriered Animal Breeding Unit, Alderley Park, Cheshire, UK.

On arrival the mice and rats were housed by species with up to 5 per cage on mobile mouse or rat cage racks and given food, PCD or CT1 (supplied by Special Diets Services, Stepfield, Witham, Essex, UK; Appendix A) and filtered tap water (via an automatic water system) ad libitum.

The animal rooms used for Phases I, II and III were maintained within a temperature range of 19-23°C, and within a relative humidity range of 40-70%. Temperature and relative humidity were monitored, using thermohygrograph charts or the Honeywell Site Monitoring System. Lighting was controlled to provide 12 hours artificial light followed by 12 hours darkness. The animal rooms were under positive pressure with respect to the access corridor and had approximately 25-30 air changes per hour.

2.5 Test Method

2.5.1 Study Design: For both mice and rats, Phase I involved the determination of a maximum tolerated dose (MTD), based on patterns of lethalities or severe toxicity observed over a three to four day observation period following a single oral dose as shown in Appendix B.

After acclimatisation, the mice and rats for Phase II were randomly distributed on to racks according to the rack plans in Appendix C. The animals were identified by cage cards and by ear punching.

In Phase II (first mouse and rat studies), male mice and rats were weighed and given a single oral dose of corn oil, cyclophosphamide (65mg/kg for mice; 20mg/kg for rats) or CI Solvent Yellow 14 at a dose level of 5000mg/kg bodyweight as detailed in Appendix D. The bodyweights were recorded prior to dosing and are detailed in Appendix H.

In Phase III (second mouse and rat studies), the mice and rats, after acclimatisation, were randomly distributed on to racks according to the rack plan in Appendix C. The animals were identified by cage cards and by ear punching. The male mice and rats were weighed and given a single oral dose of corn oil, cyclophosphamide (65mg/kg for mice; 20mg/kg for rats) or CI Solvent Yellow 14 at dose levels of 2000mg/kg (mice only) and 5000mg/kg (rats and mice) as detailed in Appendix D. The bodyweights were recorded prior to dosing and are detailed in Appendix H.

2.5.2 Summary of Methodology: Bone marrow smears were prepared 24 and 48 hours after dosing for the vehicle control and CI Solvent Yellow 14 treated animals and 24 hours after dosing for the cyclophosphamide treated animals. The preparations from the mice were stained with polychrome methylene blue and eosin to visualise the various cell types. The rat preparations were stained with haematoxylin and eosin to visualise the various cell types and to overcome any artefactual problem with the staining of mast cell granules (Pascoe and Gatehouse, 1986). Initially, one thousand polychromatic erythrocytes per slide were evaluated for the presence of micronuclei from each sample. Additional analyses up to

6000 polychromatic erythrocytes per animal were subsequently conducted for samples from all mice and all rats. In addition, 1000 erythrocytes were counted to determine the percentage of polychromatic erythrocytes in the total erythrocyte population. This provides an indication of any cytotoxicity in the bone marrow. Detailed methodology is shown in Appendix E.

2.6 Statistical Analyses

The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance, regarding each combination of sampling time and dose level as a separate group. The results were examined to determine whether any differences between vehicle control and CI Solvent Yellow 14 treated groups were consistent across sampling times.

The values for micronucleated polychromatic erythrocytes were transformed using a natural logarithmic transformation, to stabilise the variance, before analysis.

The extended counts were also considered by analysis of variance separately and combined with the original counts. The analysis of the combined data was carried out after calculating the average number of micronuclei per 1000 polychromatic erythrocytes.

All analyses were carried out using the GLM procedure in SAS (1985). Unbiased estimates of the group means were provided by the least square means (LSMEANS option in SAS) but for simplicity standard means are presented. Each treatment group mean was compared with the vehicle control group mean at the corresponding sampling time using a one-sided Student's t-test based on the error mean square in the analysis.

The data from the second mouse and second rat studies (Phase III) were analysed as separate studies.

3. RESULTS

3.1 Phase I - MTD Determination

Groups of 5 male rats and 5 male mice were dosed with CI Solvent Yellow 14 at 5000mg/kg. No lethalities and no significant clinical observations indicative of systemic toxicity were observed in either the mice or the rats and therefore 5000mg/kg was selected as the maximum tolerated dose for both mice and rats.

Orange colouration of the urine and faeces from all animals was observed.

3.2 Phase II - Micronucleus Test

The data for individual animals are shown in Appendices F and G and the group data are summarised in Tables 1-16.

No significant adverse reactions to treatment were observed for either mice or rats dosed with CI Solvent Yellow 14. Clinical signs observed included orange colouration of the urine, faeces, fur, feet and tails and a slightly subdued nature.

Considering firstly the rat study, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 and 48 hour sampling times in animals dosed with CI Solvent Yellow 14 at 5000mg/kg. A small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was also observed at the 48 hour sampling time in extended counts of 2000 polychromatic erythrocytes per animal. Small but statistically significant increases were observed at the 24 and 48 hour sampling times when the original and extended counts were combined prior to statistical analysis.

In the second rat study, small increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 and 48 hour sampling times in animals dosed with CI Solvent Yellow 14 at 5000mg/kg, the increase at the 24 hour sampling time reaching statistical significance.

To further investigate the small increases observed in both rat studies, extended analysis of the slides from all animals was conducted to increase the database to a total of 6000 polychromatic erythrocytes per animal.

Considering the data from the combined counts of 6000 polychromatic erythrocytes per animal, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 and 48 hour sampling times in both studies (Table 11 and 12).

Comparison of the percentage of polychromatic erythrocytes showed no statistically significant differences between the CI Solvent Yellow 14 and the vehicle control animals in either study with the exception of the rats dosed at 5000mg/kg and sampled at 48 hours in the second study. The decrease observed in these animals is very small and is considered not to be of biological significance.

In the first mouse study, a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was observed at the 48 hour sampling time in animals dosed with CI Solvent Yellow 14 at 5000mg/kg. A small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was also observed at the 48 hour sampling time in extended counts of 2000 polychromatic erythrocytes per animal and when the original and extended counts were combined prior to statistical analysis.

In the second mouse study, small increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 hour sampling time in animals dosed with CI Solvent Yellow 14 at 2000 and 5000mg/kg, the increase in mice dosed at 5000mg/kg

reaching statistical significance.

To further investigate the small increases observed in both mouse studies, extended analysis of the slides from all animals was conducted to increase the database to a total of 6000 polychromatic erythrocytes per animal.

Considering the data from the combined counts of 6000 polychromatic erythrocytes per animal, a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was observed in the first study at the 48 hour sampling time in mice dosed with CI Solvent Yellow 14 at 5000mg/kg (Table 5).

In the second study, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed at the 24 hour sampling time in mice dosed with CI Solvent Yellow 14 at 2000mg/kg and at the 24 and 48 hour sampling times in mice dosed with CI Solvent Yellow 14 at 5000mg/kg (Table 6). A small increase was observed at the 48 hour sampling time in mice treated with CI Solvent Yellow 14 at 2000mg/kg but this did not reach statistical significance.

Comparison of the percentage of polychromatic erythrocytes showed no statistically significant differences between the CI Solvent Yellow 14 and the vehicle control animals in either study with the exception of the mice dosed at 2000mg/kg and sampled at 24 hours in the second study. The decrease observed in these animals is very small and is considered not to be of any biological significance.

The test system positive control, cyclophosphamide, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to the vehicle control values, in both rat and both mouse studies, thus demonstrating the sensitivity of the test system to a known clastogen.

4. DISCUSSION

The literature report by Westmoreland and Gatehouse (1991) was of significance in that it claimed an *in vivo* genotoxic response for Solvent Yellow 14 in the rat, and also that the effect was species specific in that the mouse showed no such response. In order to evaluate these observations, using a sample of CI Solvent Yellow 14 from Zeneca Specialties, micronucleus tests were conducted at CTL in both the rat and mouse.

The criteria for a valid test system as laid down by OECD Guideline 474 (1983) for the conduct of micronucleus studies, are that the positive control substance should induce a significant elevation in micronucleated polychromatic erythrocytes compared to the vehicle control values, and that the test material should be tested at a level that causes a decrease in the percentage of polychromatic erythrocytes (indicating a cytotoxic effect on the bone marrow) or at the maximum tolerated dose level.

The studies satisfy these criteria in that CI Solvent Yellow 14 was tested at the limit dose for the assay. The positive control substance, cyclophosphamide, gave statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to vehicle control values in both rat and both mouse studies.

Initial evaluation of the incidence of micronucleated polychromatic erythrocytes observed in 1000 polychromatic erythrocytes per animal in both the rats and mice resulted in some increases over the vehicle control values. Analysis of increased numbers of polychromatic erythrocytes together with a repeat evaluation in both the rat (5000mg/kg) and mouse (2000 and 5000mg/kg) were undertaken in order to clarify these findings. The final interpretation was made from an examination of 6000 polychromatic erythrocytes from all animals studied.

Firstly considering the rat, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the

vehicle control values, were observed in both studies. Although the increases were small they were consistently observed over the large database of 6000 polychromatic erythrocytes per animal and were reproducible in the two rat studies. These data, in agreement with those of Westmoreland and Gatehouse (1991), indicate that CI Solvent Yellow 14 is clastogenic in the rat bone marrow micronucleus test following administration via the oral route.

Colouration of the urine of all rats dosed with CI Solvent Yellow 14 was observed in the CTL studies indicating that the test material was absorbed and distributed following oral administration. Westmoreland and Gatehouse (1991) reported yellowing of the fatty tissues in the rats in their studies. This was not observed in the CTL studies, but it is difficult to see how any such colouration, given the yellow colour of the test material, would be discernable from the yellow fat colour observed in control animals.

In the mouse, small but statistically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed in both studies. Although the magnitude of these increases was smaller than those observed in the rat studies, they were consistently observed over the large database of 6000 polychromatic erythrocytes per animal and were reproducible in the two studies. The data indicate that CI Solvent Yellow 14 is resulting in the induction of micronuclei in the bone marrow of mice following oral dosing at 5000mg/kg. This weakly clastogenic effect of CI Solvent Yellow 14 is not in agreement with the published results of Westmoreland and Gatehouse (1991) who tested Solvent Yellow 14 up to 2000mg/kg in one study using male CRH mice. Westmoreland and Gatehouse (1991) did not observe any statistically significant increases in the incidence of micronucleated polychromatic erythrocytes following analysis of 2000 polychromatic erythrocytes per animal. As observed in our studies they also did not observe any significant cytotoxic effects on the bone marrow.

There are several differences between the CTL studies and the Westmoreland and Gatehouse study including, strain of mouse (C57BL/6J$_f$BL10/Alpk vs CRH)

and rat (Alpk:AP$_f$SD vs PVG), number of cells analysed, source of material (Zeneca Specialties vs NTP Repository and Simga), dose level used and absorption of the test material. These latter two points are considered to be the most likely explanations for the apparent discrepancies between the results of the mouse studies. The CTL studies used a top dose level of 5000mg/kg, whereas the Westmoreland and Gatehouse mouse study stopped at 2000mg/kg. However, a small effect was observed even at 2000mg/kg in the CTL study. Regarding the absorption of the test material, Westmoreland and Gatehouse (1991) reported that there was not any visible evidence of absorption into the fatty tissues of mice as evidenced by yellowing of those tissues, whereas in the CTL study the orange colouration of the urine observed from all mice dosed with CI Solvent Yellow 14 clearly indicates systemic absorption of the material following oral dosing. Colouration of the fatty tissues was not observed in the mice from the CTL study, but as discussed for the rat, it is difficult to see how any such colouration, due to the yellow colour of the test material, would be discernible from the yellow fat colour observed in control animals.

It can be concluded therefore that CI Solvent Yellow 14 is absorbed systemically in both rats and mice dosed orally with CI Solvent Yellow 14 and that the apparent absorption difference observed between rats and mice by Westmoreland and Gatehouse (1991) has not been confirmed in our studies.

It can also be concluded that CI Solvent Yellow 14 is, as previously reported by Westmoreland and Gatehouse (1991), clastogenic in the rat bone marrow causing increases in the incidence of polychromatic erythrocytes. In addition, CI Solvent Yellow 14 has been shown to be weakly clastogenic in the mouse bone marrow and therefore the species specificity reported by Westmoreland and Gatehouse (1991) has not been confirmed.

5. CONCLUSION

Considering all the data from the mouse and rat studies, it is concluded that CI Solvent Yellow 14, under the conditions of test, is clastogenic in the rat micronucleus test and weakly clastogenic in the mouse micronucleus test.

6. REFERENCES

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TABLE 1

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Mouse Study - Original Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	1.8 ± 1.9	1.4 ± 1.1
12	Cyclophosphamide	65mg/kg	17.0 ± 6.3**	
13	CI Solvent Yellow 14	5000mg/kg	2.8 ± 2.3	5.0 ± 2.9**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on 1000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 2

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Mouse Study - Extended Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	2.4 ± 1.1	1.6 ± 1.3
12	Cyclophosphamide	65mg/kg	10.6 ± 5.3**	
13	CI Solvent Yellow 14	5000mg/kg	3.0 ± 1.2	4.0 ± 1.2**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on an additional 2000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 3

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Mouse Study - Combined Original and Extended Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	2.1 ± 1.1	1.5 ± 0.8
12	Cyclophosphamide	65mg/kg	13.8 ± 4.4**	
13	CI Solvent Yellow 14	5000mg/kg	2.9 ± 1.4	4.5 ± 1.8**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on a total of 3000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 4

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Mouse Study - Original Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
18	Vehicle Control (Corn Oil)	20ml/kg	3.8 ± 1.6	2.4 ± 2.5
19	Cyclophosphamide	65mg/kg	27.2 ± 5.4**	
20	CI Solvent Yellow 14	2000mg/kg	5.4 ± 2.7	3.2 ± 0.8
21	CI Solvent Yellow 14	5000mg/kg	7.0 ± 3.1*	2.2 ± 0.5

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

\+ Based on 1000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 5

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Mouse Study - Total Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	2.4 ± 0.4	1.5 ± 0.7
12	Cyclophosphamide	65mg/kg	14.9 ± 3.1**	
13	CI Solvent Yellow 14	5000mg/kg	2.9 ± 1.0	4.6 ± 0.8**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on a total of 6000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 6

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Mouse Study - Total Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
18	Vehicle Control (Corn Oil)	20ml/kg	2.3 ± 0.4	2.1 ± 0.5
19	Cyclophosphamide	65mg/kg	21.7 ± 3.1**	
20	CI Solvent Yellow 14	2000mg/kg	3.8 ± 1.0**	2.9 ± 1.0
21	CI Solvent Yellow 14	5000mg/kg	4.4 ± 1.4**	3.1 ± 1.3*

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

\+ Based on a total of 6000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 7

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Rat Study - Original Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	0.6 ± 0.9	0.0 ± 0.0
12	Cyclophosphamide	20mg/kg	35.6 ± 5.6**	
13	CI Solvent Yellow 14	5000mg/kg	7.8 ± 7.4**	3.6 ± 2.5**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on 1000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 8

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Rat Study - Extended Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	1.2 ± 0.8	0.0 ± 0.0
12	Cyclophosphamide	20mg/kg	29.4 ± 8.4**	
13	CI Solvent Yellow 14	5000mg/kg	2.4 ± 3.2	1.8 ± 2.1*

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

\+ Based on extended analysis of 2000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 9

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Rat Study - Combined Original and Extended Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	0.9 ± 0.7	0.0 ± 0.0
12	Cyclophosphamide	20mg/kg	32.5 ± 6.4**	
13	CI Solvent Yellow 14	5000mg/kg	5.1 ± 5.0**	2.7 ± 1.6**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on a total of 3000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 10

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Rat Study - Original Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
17	Vehicle Control (Corn Oil)	20ml/kg	0.0 ± 0.0	0.2 ± 0.5
18	Cyclophosphamide	20mg/kg	14.4 ± 8.0**	
19	CI Solvent Yellow 14	5000mg/kg	1.8 ± 2.5*	1.6 ± 2.1

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

* Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided) on transformed data.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

\+ Based on 1000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 11

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Rat Study - Total Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	0.4 ± 0.3	0.5 ± 0.4
12	Cyclophosphamide	20mg/kg	31.6 ± 7.0**	
13	CI Solvent Yellow 14	5000mg/kg	3.8 ± 3.1**	3.2 ± 1.7**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on a total of 6000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 12

MEAN INCIDENCE OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES ± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Rat Study - Total Counts+

Group	Compound	Dose	Mean Incidence of MPE/1000 PE ± SD	
			24 hours	48 hours
17	Vehicle Control (Corn Oil)	20ml/kg	0.2 ± 0.2	0.1 ± 0.1
18	Cyclophosphamide	20mg/kg	23.1 ± 10.7**	
19	CI Solvent Yellow 14	5000mg/kg	1.9 ± 1.5**	3.9 ± 1.7**

PE = polychromatic erythrocytes.
MPE = micronucleated polychromatic erythrocytes.
SD = standard deviation.

** Statistically significant increase in micronucleated polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided) on transformed data.

+ Based on a total of 6000 polychromatic erythrocytes per animal.

All means based on 5 animals.

TABLE 13

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Mouse Study

Group	Compound	Dose	Mean % Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	39.6 ± 2.1	40.3 ± 2.2
12	Cyclophosphamide	65mg/kg	40.6 ± 1.7	
13	CI Solvent Yellow 14	5000mg/kg	39.9 ± 1.7	40.0 ± 2.3

SD = standard deviation.

All means based on 5 animals.

TABLE 14

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Mouse Study

Group	Compound	Dose	Mean % Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
18	Vehicle Control (Corn Oil)	20ml/kg	46.7 ± 4.2	44.9 ± 1.1
19	Cyclophosphamide	65mg/kg	39.8 ± 3.4**	
20	CI Solvent Yellow 14	2000mg/kg	42.7 ± 4.1*	42.2 ± 3.7
21	CI Solvent Yellow 14	5000mg/kg	46.3 ± 2.3	42.8 ± 3.3

SD = standard deviation.

* Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided).

** Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided).

All means based on 5 animals.

TABLE 15

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

First Rat Study

Group	Compound	Dose	Mean % Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	44.9 ± 1.5	41.1 ± 1.4
12	Cyclophosphamide	20mg/kg	39.2 ± 2.8**	
13	CI Solvent Yellow 14	5000mg/kg	43.1 ± 5.2	39.8 ± 1.2

SD = standard deviation.

** Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.01$ in the Student's 't' test (one-sided).

All means based on 5 animals.

TABLE 16

MEAN PERCENTAGE OF POLYCHROMATIC ERYTHROCYTES
± STANDARD DEVIATION (SD) AT TWO SAMPLING TIMES

GROUP MEAN ANIMAL DATA - MALES

Second Rat Study

Group	Compound	Dose	Mean % Polychromatic Erythrocytes ± SD	
			24 hours	48 hours
17	Vehicle Control (Corn Oil)	20ml/kg	40.9 ± 3.0	43.0 ± 1.4
18	Cyclophosphamide	20mg/kg	36.4 ± 3.7*	
19	CI Solvent Yellow 14	5000mg/kg	41.4 ± 3.8	37.9 ± 5.8*

SD = standard deviation.

* Statistically significant decrease in the percentage of polychromatic erythrocytes at $p<0.05$ in the Student's 't' test (one-sided).

All means based on 5 animals.

APPENDIX A

COMPOSITION OF CT1 DIET

Manufacturer - Special Diets Services Ltd, Stepfield, Witham, Essex, UK.

Dietary constituents and a proximate analysis are given below. The diet is prepared to a constant formula, details of which are available on request.

Dietary Constituents	Proximate Analysis	%
Wheat	Crude protein	20.0
Wheat feed	Crude oil	3.4
Wheat bran	Crude fibre	3.0
Maize	Moisture	9.0
Cornflour	Ash	6.0
Soya bean meal extract	Calcium	0.96
British white fish meal	Phosphorus	0.93
Skim milk powder (spray dried)		
PCD vitamin and mineral premix		

All batches of CT1 diet complied with the following contaminants specification:

Chemical Contaminant	Maximum Permitted Concentration (ppm)	Microbiological Contaminant	Maximum Permitted
Arsenic	1.0	Total viable organisms	2×10^4/ g
Cadmium	0.5		
Lead	3.0		
Mercury	0.1	Mesophilic spores	2×10^4/ g
Selenium	0.5		
DDT (total)	0.1	Salmonella sp	None / g
Dieldrin	0.02		
Heptachlor	0.01	Faecal E coli (Type 1)	None / g
Lindane	0.1		
PCB's (total)	0.05		
Fluorine	40	Coliforms	None / g
Nitrite	5.0	Fungal units	200 / g
Nitrate	100		
Aflatoxins (total)	0.001	Antibiotic activity	None / g
Malathion	0.5		

COMPOSITION OF PORTON COMBINED DIET (PCD)

Manufacturer - Special Diets Services Ltd, Stepfield, Witham, Essex, UK.

Dietary constituents and a proximate analysis are given below. The diet is prepared to a constant formula, details of which are available on request.

Dietary Constituents
Wheat
Wheat feed
Oats
Maize
Barley
Soya bean meal extract
British white fish meal
Skim milk powder (spray dried)
Yeast (unextracted)
PCD vitamin and mineral premix

Proximate Analysis (all values calculated to nominal 10% moisture content)	%
Crude protein	20.0
Crude oil	3.0
Crude fibre	5.0
Ash	6.9
Calcium	0.94
Phosphorus	0.80

All batches of PCD diet complied with the following contaminants specification:

Chemical Contaminant	Maximum Permitted Concentration (ppm)	Microbiological Contaminant	Maximum Permitted
Arsenic	1.0	Total viable organisms	2×10^4/ g
Cadmium	0.5		
Lead	3.0		
Mercury	0.1	Mesophilic spores	2×10^4/ g
Selenium	0.5		
DDT (total)	0.1	Salmonella sp	None / g
Dieldrin	0.02		
Heptachlor	0.01	Faecal E coli (Type 1)	None / g
Lindane	0.1		
PCB's (total)	0.05		
		Coliforms	None / g
Fluorine	40		
Nitrite	5.0	Fungal units	200 / g
Nitrate	100		
		Antibiotic activity	None / g
Aflatoxins (total)	0.001		
Malathion	0.5		

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX B

COMPOUND ADMINISTRATION : MTD DETERMINATION

CI Solvent Yellow 14 was administered as a single oral dose to groups of 5 male rats and 5 male mice at a dose level of 5000mg/kg. The results are shown below:-

Group	Compound	Dose (mg/kg)	Sex	Animal Number	No. of deaths /No. dosed
1M	CI Solvent Yellow 14	5000	Male	1-5	0/5
1R	CI Solvent Yellow 14	5000	Male	1-5	0/5

M = mouse
R = rat

The maximum tolerated dose (MTD) was selected as 5000mg/kg for both mice and rats.

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX C

RACK PLANS - PHASE II

First Mouse Study/First Rat Study

Males 24h Kill	76-80 (12)	81-85 (13)	71-75 (11)	Spares (13)
Males 48h Kill	91-95 (13)	86-90 (11)		

Group 11 = Vehicle control - 20ml/kg
Group 12 = Cyclophosphamide - 65mg/kg (mouse); 20mg/kg (rat)
Group 13 = CI Solvent Yellow 14 - 5000mg/kg

Group numbers are shown in parentheses.

h = hour

APPENDIX C - continued

RACK PLANS - PHASE III

Second Mouse Study

Males 24h Kill	136-140 (19)	146-150 (21)	131-135 (18)	141-145 (20)
Males 48h Kill	161-165 (21)	151-155 (18)	156-160 (20)	

Group 18 = Vehicle control - 20ml/kg
Group 19 = Cyclophosphamide - 65mg/kg
Group 20 = CI Solvent Yellow 14 - 2000mg/kg
Group 21 = CI Solvent Yellow 14 - 5000mg/kg

Second Rat Study

Males 24h Kill	126-130 (18)	131-135 (19)	121-125 (17)
Males 48h Kill	141-145 (19)	136-140 (17)	

Group 17 = Vehicle control - 20ml/kg
Group 18 = Cyclophosphamide - 20mg/kg
Group 19 = CI Solvent Yellow 14 - 5000mg/kg

Group numbers are shown in parentheses.

h = hour

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX D

ANIMAL ALLOCATION TO DOSING GROUPS - PHASE II

First Mouse Study/First Rat Study

Group	Compound	Dose	Sex	Animal Numbers/Time of Kill	
				24 hours	48 hours
11	Vehicle Control (Corn Oil)	20ml/kg	M	71-75	86-90
12	Cyclophosphamide	65mg/kg (mouse) 20mg/kg (rat)	M	76-80	
13	CI Solvent Yellow 14	5000mg/kg	M	81-85	91-95

M = male

APPENDIX D - continued

ANIMAL ALLOCATION TO DOSING GROUPS - PHASE III

Second Mouse Study

Group	Compound	Dose	Sex	Animal Numbers/Time of Kill	
				24 hours	48 hours
18	Vehicle Control (Corn Oil)	20ml/kg	M	131-135	151-155
19	Cyclophosphamide	65mg/kg	M	136-140	
20	CI Solvent Yellow 14	2000mg/kg	M	141-145	156-160
21	CI Solvent Yellow 14	5000mg/kg	M	146-150	161-165

Second Rat Study

Group	Compound	Dose	Sex	Animal Numbers/Time of Kill	
				24 hours	48 hours
17	Vehicle Control (Corn Oil)	20ml/kg	M	121-125	136-140
18	Cyclophosphamide	20mg/kg	M	126-130	
19	CI Solvent Yellow 14	5000mg/kg	M	131-135	141-145

M = male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX E

PROCESSING OF BONE MARROW AND CRITERIA FOR IDENTIFICATION OF MICRONUCLEI

The animals were killed by asphyxiation in halothane Ph. Eur. (FLUOTHANE, ICI Pharmaceuticals PLC), or in a rising concentration of carbon dioxide followed by cervical dislocation 24 and 48 hours after receiving a single oral dose of the test material.

a) Femurs were removed and stripped clean of muscle.

b) The iliac end of the femur was removed and a fine paint brush was rinsed in saline, wiped to remove the excess and wetted with a solution of albumin (6% w/v in physiological saline). This was then dipped into the marrow canal and two smears were painted on an appropriately labelled clean, dry microscope slide. This procedure was repeated to give four smears of marrow per slide. The brush was rinsed in physiological saline between animals of the same group, and a separate brush and pot of physiological saline were used between groups to avoid cross contamination.

c) The slides were allowed to air dry.

d) The slides from the mice were then stained with polychrome methylene blue and eosin using an Ames Hema-Tek staining machine (Hema-Tek, Miles Laboratory Limited, Stoke Court, Stoke Poges, Slough, Berkshire, UK). The slides for the rats were stained manually with haematoxylin and eosin.

e) Slides were coded and scored blind, in numerical slide code order.

APPENDIX E - continued

PROCESSING OF BONE MARROW AND CRITERIA FOR
IDENTIFICATION OF MICRONUCLEI

f) Initially, one thousand polychromatic erythrocytes were examined for the presence of micronuclei using x10 or x12.5 eye pieces and a x100 oil immersion objective lens for each animal. Extended analysis up to 6000 polychromatic erythrocytes per animal was subsequently conducted for all animals. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of polychromatic to normochromatic erythrocytes in a sample of 1000 erythrocytes.

Criteria for identification of micronuclei are as described by Schmid (1976):

(i) Spherical (or rounded) with well-defined edges.
(ii) Diameters of not less than approximately 1/20 of a polychromatic erythrocyte diameter.
(iii) Dark purple/dark blue staining.
(iv) Lie in the same plane as the polychromatic erythrocyte in which it is contained (determined by focusing).

Reference:

Schmid W (1976). The Micronucleus Test for Cytogenetic Analysis. In: A Hollaender (Ed). Chemical Mutagens : Principles and Methods For Their Detection. Vol 4, Plenum, New York 31-43.

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Original Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	0	2	1	5	1	2	0	1	1	3
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	10	24	22	11	18					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	0	2	2	4	6	9	4	2	3	7

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS						48 HRS					
Extended Count No.				1						1					
11	CORN OIL	20 ML/KG	M	1	2	3	2	4	1	1	1	4	1
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	5	9	8	12	19					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	2	5	3	3	4	4	2	5	5

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Extended Counts

Group	Compound	Extended Count No. 2 Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	3	2	3	3	4	1	1	0	2	0
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	20	12	10	8	27					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	0	2	3	2	6	4	5	2	5

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 3													
11	CORN OIL	20 ML/KG	M	5	1	1	3	2	3	2	3	3	1
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	19	18	16	12	20					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	6	0	2	3	5	1	1	1	5	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 4													
11	CORN OIL	20 ML/KG	M	6	2	4	0	1	1	1	1	3	0
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	13	14	15	19	23					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	4	3	2	7	3	7	6	11	12	4

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study - Extended Counts

Extended Count No. 5

Group	Compound	Dose	Sex	24 HRS						48 HRS				
11	CORN OIL	20 ML/KG	M	1	2	1	4	3	4	1	2	1	0	
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	12	9	13	14	15						
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	5	1	3	4	2	3	3	6	7	3	

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Original Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
18	CORN OIL	20 ML/KG	M	4	5	4	5	1	1	0	6	4	1
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	27	28	34	19	28					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	8	3	7	2	7	4	4	2	3	3
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	7	2	10	9	7	3	2	2	2	2

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 1													
18	CORN OIL	20 ML/KG	M	3	3	2	4	4	0	2	3	1	2
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	21	24	21	20	27					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	5	3	6	4	2	5	5	1	3	1
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	4	4	7	3	2	6	0	3	4	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 2										
				24 HRS					48 HRS					
18	CORN OIL	20 ML/KG	M	0	1	1	5	0	3	1	1	5	2
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	23	22	20	12	22					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	4	2	5	6	0	3	3	3	3	1
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	4	5	5	6	7	3	3	4	3	4

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 3									
				24 HRS						48 HRS			
18	CORN OIL	20 ML/KG	M	4	2	4	2	1	3	1	0	2	0
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	14	15	16	13	15					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	3	1	2	3	3	3	2	2	0	5
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	3	1	4	11	4	6	3	1	2	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 4										
				24 HRS					48 HRS					
18	CORN OIL	20 ML/KG	M	2	1	2	2	5	5	5	4	1	1
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	18	25	27	16	28					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	4	4	6	2	2	1	5	2	2	1
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	2	1	3	2	7	1	2	7	3

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 5										
				24 HRS						48 HRS				
18	CORN OIL	20 ML/KG	M	0	0	1	0	2	1	2	0	3	2
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	21	29	15	22	29					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	4	3	3	2	7	1	8	6	2	2
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	4	3	4	6	1	5	2	1	6	5

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Original Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	0	1	0	0	2	0	0	0	0	0
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	32	43	29	35	39					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	6	19	11	1	8	3	2	2	3

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No.				1					2				
11	CORN OIL	20 ML/KG	M	1	0	1	2	2	0	0	0	0	0
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	34	33	17	25	38					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	1	8	0	1	2	2	0	5	0

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 2													
11	CORN OIL	20 ML/KG	M	0	0	0	0	0	0	0	1	2	0
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	16	26	29	35	48					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	3	2	4	4	1	6	0	5	3	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Extended Counts

Group	Compound	Extended Count No. 3 Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	0	0	0	2	0	3	0	0	0	0
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	21	35	25	39	34					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	1	7	4	1	8	4	5	3	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 4													
11	CORN OIL	20 ML/KG	M	0	0	0	0	0	1	1	0	2	0
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	22	30	21	42	24					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	6	7	5	3	5	5	1	5	2

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study - Extended Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
Extended Count No. 5													
11	CORN OIL	20 ML/KG	M	0	0	0	0	0	2	1	1	1	1
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	23	43	19	51	40					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	1	9	1	1	6	1	1	5	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Original Counts

Group	Compound	Dose	Sex	24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	0	0	0	0	0	0	1	0	0	0
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	20	2	21	11	18					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	0	1	0	6	2	5	2	0	1	0

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Extended Counts

Group	Compound	Extended Count No. 1 Dose	Sex	24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	0	0	0	0	0	0	0	0	0	1
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	35	10	29	15	31					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	0	2	3	2	6	3	3	7	1

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 2 24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	1	0	0	0	0	0	0	0	0	0
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	36	17	27	14	23					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	3	0	0	9	6	4	2	6	0

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Extended Counts

Group	Compound	Extended Count No. 3 Dose	Sex	24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	0	0	1	0	1	0	0	0	0	0
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	51	15	37	20	14					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	0	0	1	2	2	1	4	6	4	2

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 4 24 HRS					Extended Count No. 4 48 HRS				
17	CORN OIL	20 ML/KG	M	0	0	0	0	1	1	0	0	0	0
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	36	16	44	15	8					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	1	2	0	6	4	8	6	3	8	2

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX F - continued

INDIVIDUAL ANIMAL DATA
MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES/1000 POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study - Extended Counts

Group	Compound	Dose	Sex	Extended Count No. 5									
				24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	1	0	1	0	0	0	0	0	0	0
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	36	15	43	21	13					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	2	1	1	3	3	7	6	6	5	3

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX G

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

PHASE II

First Mouse Study

Group	Compound	Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	37.4	42.3	39.4	37.8	41.3	38.1	42.6	40.3	42.4	38.1
12	CYCLOPHOSPHAMIDE	65 MG/KG	M	43.3	41.0	39.5	39.9	39.3					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	39.3	42.8	39.7	39.0	38.6	40.2	37.1	38.7	43.3	40.5

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Mouse Study

Group	Compound	Dose	Sex	24 HRS					48 HRS				
18	CORN OIL	20 ML/KG	M	44.1	50.7	48.3	49.8	40.8	45.1	45.1	45.4	45.8	42.9
19	CYCLOPHOSPHAMIDE	65 MG/KG	M	37.9	39.0	42.2	35.8	44.2					
20	CI SOLVENT YELLOW 14	2000 MG/KG	M	44.8	42.5	43.8	46.5	35.9	45.0	38.6	37.8	44.7	45.0
21	CI SOLVENT YELLOW 14	5000 MG/KG	M	48.2	44.9	42.9	47.7	47.8	45.7	37.2	43.8	43.4	43.9

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

PHASE II

First Rat Study

Group	Compound	Dose	Sex	24 HRS					48 HRS				
11	CORN OIL	20 ML/KG	M	43.8	46.2	45.1	42.9	46.3	39.3	41.5	42.7	41.8	40.2
12	CYCLOPHOSPHAMIDE	20 MG/KG	M	34.3	39.8	40.5	40.1	41.3					
13	CI SOLVENT YELLOW 14	5000 MG/KG	M	48.3	42.0	38.6	48.6	37.8	40.7	41.1	39.7	39.1	38.2

M - male

CI SOLVENT YELLOW 14: AN EVALUATION IN THE RAT AND MOUSE MICRONUCLEUS TESTS

APPENDIX G - continued

INDIVIDUAL ANIMAL DATA - % POLYCHROMATIC ERYTHROCYTES

PHASE III

Second Rat Study

Group	Compound	Dose	Sex	24 HRS					48 HRS				
17	CORN OIL	20 ML/KG	M	38.2	39.8	46.1	39.9	40.7	41.8	42.1	45.0	44.1	42.2
18	CYCLOPHOSPHAMIDE	20 MG/KG	M	39.0	35.6	38.2	39.0	30.4					
19	CI SOLVENT YELLOW 14	5000 MG/KG	M	39.9	47.3	36.8	41.8	41.4	45.0	37.7	35.7	41.4	29.6

M - male

APPENDIX H

INDIVIDUAL BODYWEIGHTS (g) - PHASE II

First Mouse Study		First Rat Study	
Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
71	20.4	71	188
72	24.3	72	196
73	23.4	73	242
74	20.4	74	200
75	21.5	75	218
76	22.5	76	205
77	22.8	77	221
78	23.6	78	181
79	22.7	79	207
80	21.9	80	194
81	24.4	81	216
82	22.6	82	193
83	-	83	212
84	23.1	84	246
85	20.2	85	228
86	22.6	86	202
87	22.7	87	231
88	23.1	88	250
89	22.2	89	238
90	22.4	90	257
91	24.2	91	198
92	22.4	92	214
93	24.3	93	221
94	20.4	94	229
95	21.6	95	215

INDIVIDUAL BODYWEIGHTS (g) - PHASE III

Second Mouse Study		Second Rat Study	
Animal Number	Bodyweight (g)	Animal Number	Bodyweight (g)
131	22.9	121	180
132	22.1	122	123
133	18.8	123	182
134	20.9	124	170
135	20.9	125	183
136	19.0	126	216
137	18.8	127	169
138	21.6	128	191
139	20.7	129	190
140	24.0	130	190
141	22.0	131	170
142	19.0	132	193
143	24.0	133	143
144	23.0	134	198
145	21.0	135	161
146	23.0	136	161
147	22.0	137	159
148	24.0	138	151
149	23.0	139	201
150	19.0	140	146
151	18.2	141	162
152	24.3	142	193
153	22.9	143	195
154	25.0	144	180
155	22.6	145	159
156	23.0		
157	18.0		
158	17.0		
159	24.0		
160	19.0		
161	21.0		
162	21.0		
163	21.0		
164	21.0		
165	20.0		

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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Stephen K. Harvey
Manager, Environment and Product Safety
ZENECA Specialties
P.O. Box 751
Wilmington, Deleware 19897

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MAY 0 3 1994

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite this number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

12728 A

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 1093 - 12728 SEQ. A

TYPE: (INT.) SUPP FLWP

SUBMITTER NAME: Zeneca Specialties

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)
DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 10/08/93 OTS DATE: 10/14/93 CSRAD DATE: 11/9/93

CHEMICAL NAME:
C.I. Solvent Yellow 14
((2-Naphthalenol, 1-(Phenylazo)-))

CAS#
~~Unknown~~
842-07-9

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
(0224)	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
(0245)	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
(0248)	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	

TRIAGE DATA: NON-CBI INVENTORY
(YES (CONTINUE))
NO (DROP)
~~DEFERRAL~~

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER:

SPECIES
RAT
MUS

TOXICOLOGICAL CONCERN:
LOW
MED
HIGH

USE: in manuf. of colored plastic materials, color gasoline

PRODUCTION:

COMMENTS: Non-Cap

11) 8EHQ-1093-12728: Rank - medium.

Chemical: CI Solvent yellow 14 (1-phenylazo-2-naphthalenol: CAS# 842-07-9).

CI solvent yellow 14: An evaluation in the rat and mouse micronucleus tests, Zeneca Central Toxicology Lab., Cheshire, UK, dated 2 July 1993: Positive for chromosome mutations (micronuclei) in the bone marrow of rats and mice exposed in vivo by oral gavage.